IN THE SPECIFICATION

Replace page 23, line 1 through page 25, line 10. Delete page 24, line 6 through page 26, line 19 and substitute therefor*:

Description of Figures

Figure 1 provides a space filling representation of the Fv fragment of the antibody 4-4-20.

Figure 2 presents the variable/constant domain interface residues for V_L (2a) and V_H (2b). For 30 non-redundant Fab fragments taken from the Brookhaven Databank, the solvent accessible surface of the amino acid side chains was calculated in the context of an Fv and of an Fab fragment. The plot shows the relative reduction in accessible surface upon contact with the constant domains (overlay plot for all 30 Fv fragments). In the sequence alignment, residues contributing to the v/c interface are highlighted. The symbols indicate the relative reduction of solvent accessible surface upon removing the constant domains (symbols: no symbol < 1%; l < 20%; n < 40%; s < 60%; t < 80%, and u ³ 80%). Circles indicate those positions which are further analyzed (see Table 1).

Figure 3 presents Western blots showing the insoluble (i) and soluble (s) fractions of cell extracts, prepared as described in Material and Methods, expressing the scFv fragments of the antibody 4-4-20. The amino acids substituted in the various mutants are given in Table 2.

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* The material to be inserted is identical to that presented in the January 15,1999 Preliminary Amendment.

Figure 4 presents a Scatchard plot of the fluorescence titration of fluorescein (20 nM) with antibody (4 to 800 nM), measured at 510 nm. The value r was obtained from $(F-F_O)/(F¥-F_O)$, where F is the measured fluorescein fluorescence at a given antibody concentration, F_O is the fluorescence in the absence of antibody and F¥ when antibody is present in large excess. Note that r gives the saturation of fluorescein by antibody. (a) Titration of wt scFv, (b) titration of Flu4 (V84D).

Figure 5 presents an overlay plot of the urea denaturation curves ((X) wt scFv, (o) Flu4).

Figure 6 presents the thermal denaturation time courses at 40 and 44°C for wt and Flu4 scFv fragment ((a) wt scFv at 40°C, (b) Flu4 at 40°C, (c) Flu4 at 44°C, (d) wt scFv at 44°C).

Table 1 describes the sequence variability of residues contributing to the v/c interface. Residue statistics are based on the variable domain sequences in the Kabat database (March 1996). Sequences which were <90% complete were excluded from the analysis. Number of sequences analyzed: human VL kappa: 404 of 881, murine VL kappa: 1061 of 2239, human VL lambda: 223 of 409, murine VL lambda: 71 of 206, human VH: 663 of 1756, murine VH: 1294 of 3849. Position refers to the sequence position according to Kabat et al. 1991, %exp. (Fab) to the relative side chain accessibility in an Fab fragment as calculated by the program NACCESS (NACCESS v2.0 by Simon Hubbard ~~(http://www.biochem.ucl.ac.uk/~roman/naccess/naccess.html)~~, %exp. (ind.) to the relative side chain accessibility in the isolated VL

or VH domain, %buried to the relative difference in side chain accessibility between Fv and Fab fragment. Consensus refers to the sequence consensus, and Distribution to the distribution of residue types.

Table 2 describes mutations introduced in the scFv fragment of the antibody 4-4-20. Each line represents a different protein carrying the mutations indicated. The residues are numbered according to Kabat et al. (1991).

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Table 3 describes K_D values of the different scFv mutants determined in fluorescence titration. The K_D values are given in nM, the error was calculated from the Scatchard analysis (Fig. 4). # determined by Miklasz et al. (1995).

The following examples illustrate the invention. --

IN THE CLAIMS

Instead of deleting "Claims" on page 33, line 1, please instead delete "Claims" on page 34, line 1 and substitute therefor -- We claim: --

REMARKS

With the exception of the formalistic corrections requested herein, applicant requests that the amendments filed in the January 15, 1999 Preliminary Amendment be entered in full. Applicants apologize for any inconvenience to the Examiner necessitated by this Supplemental Amendment.